

FIG._1A

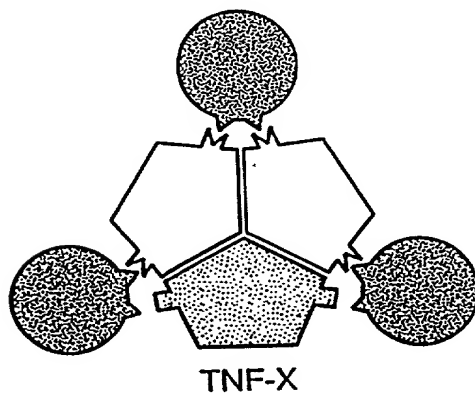


FIG._1B

TOPTOT" 682F8560

FOSTOT" 66278660

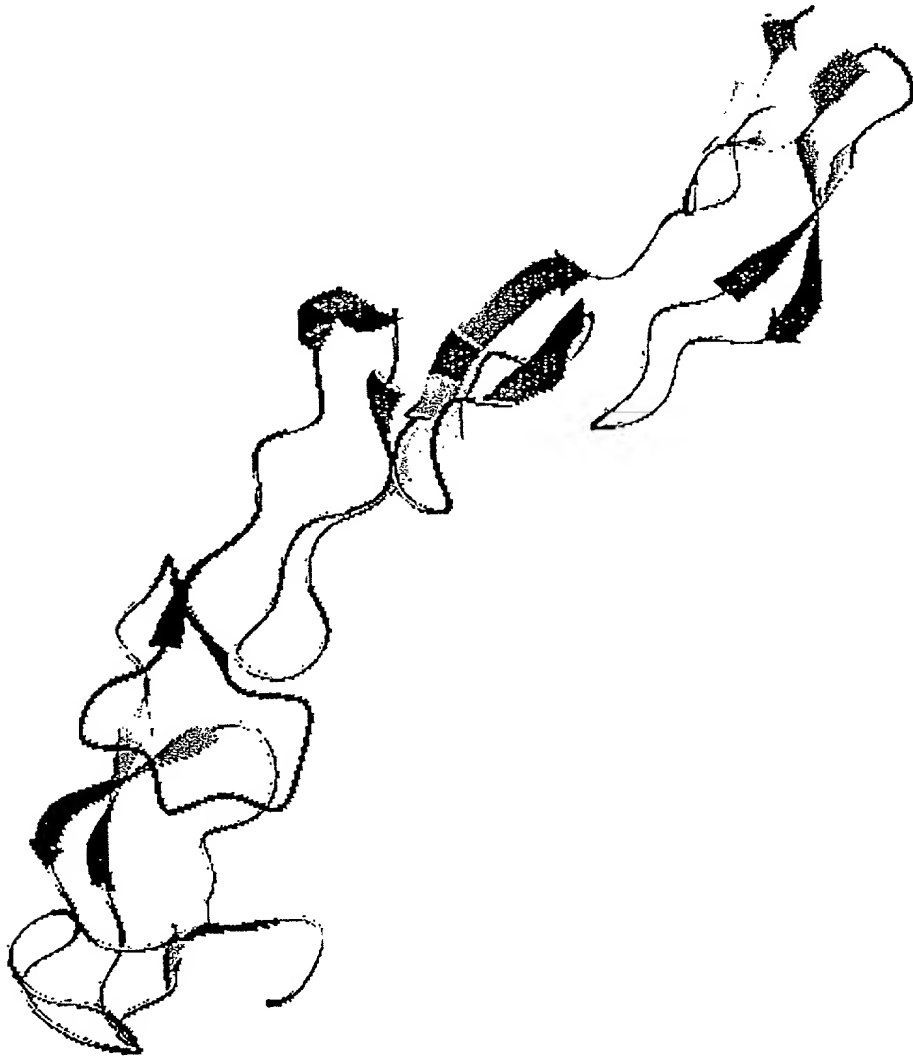
TNF-TNFR TRIMER COMPLEX

SIDE VIEW

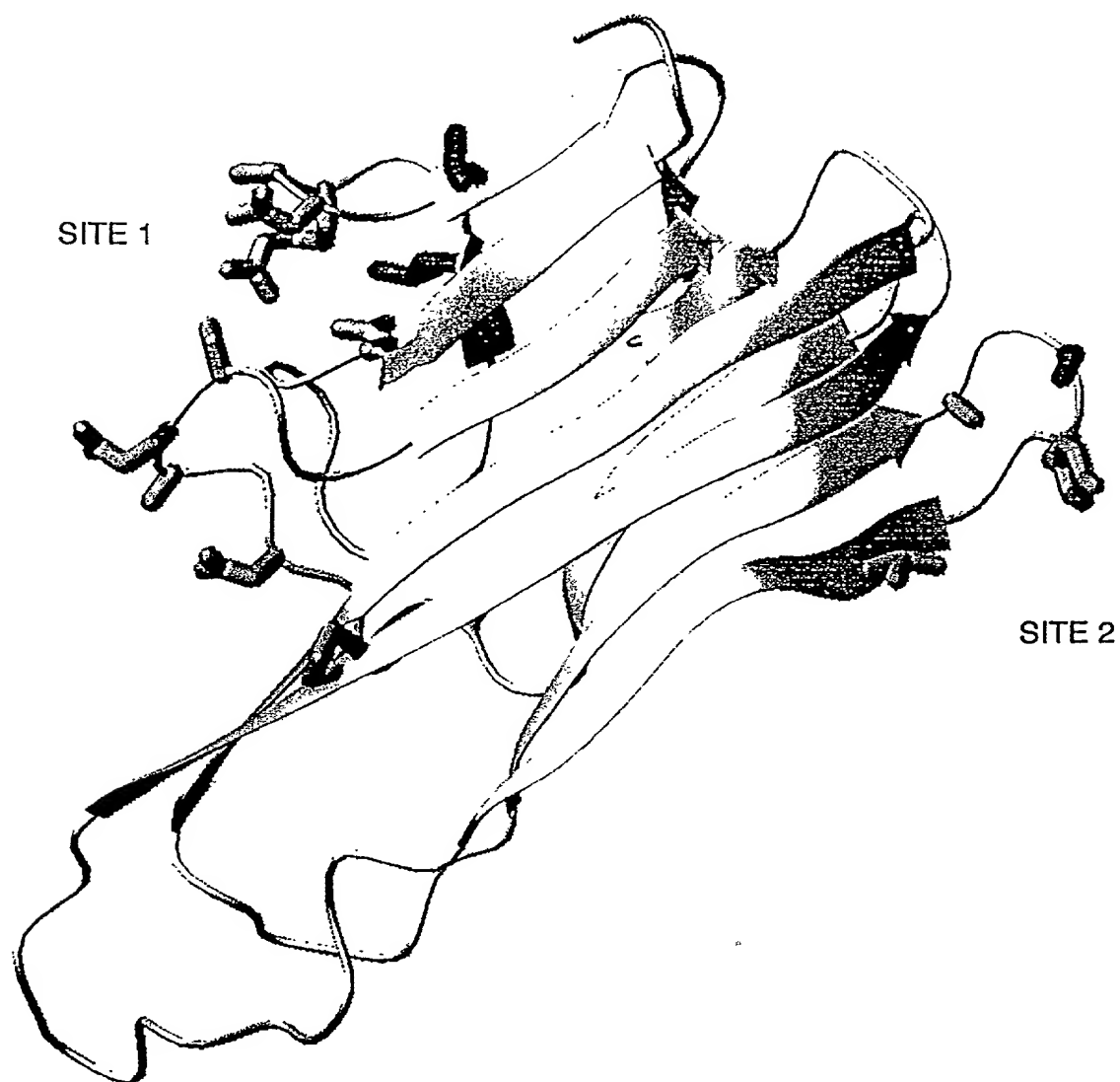
TOP VIEW

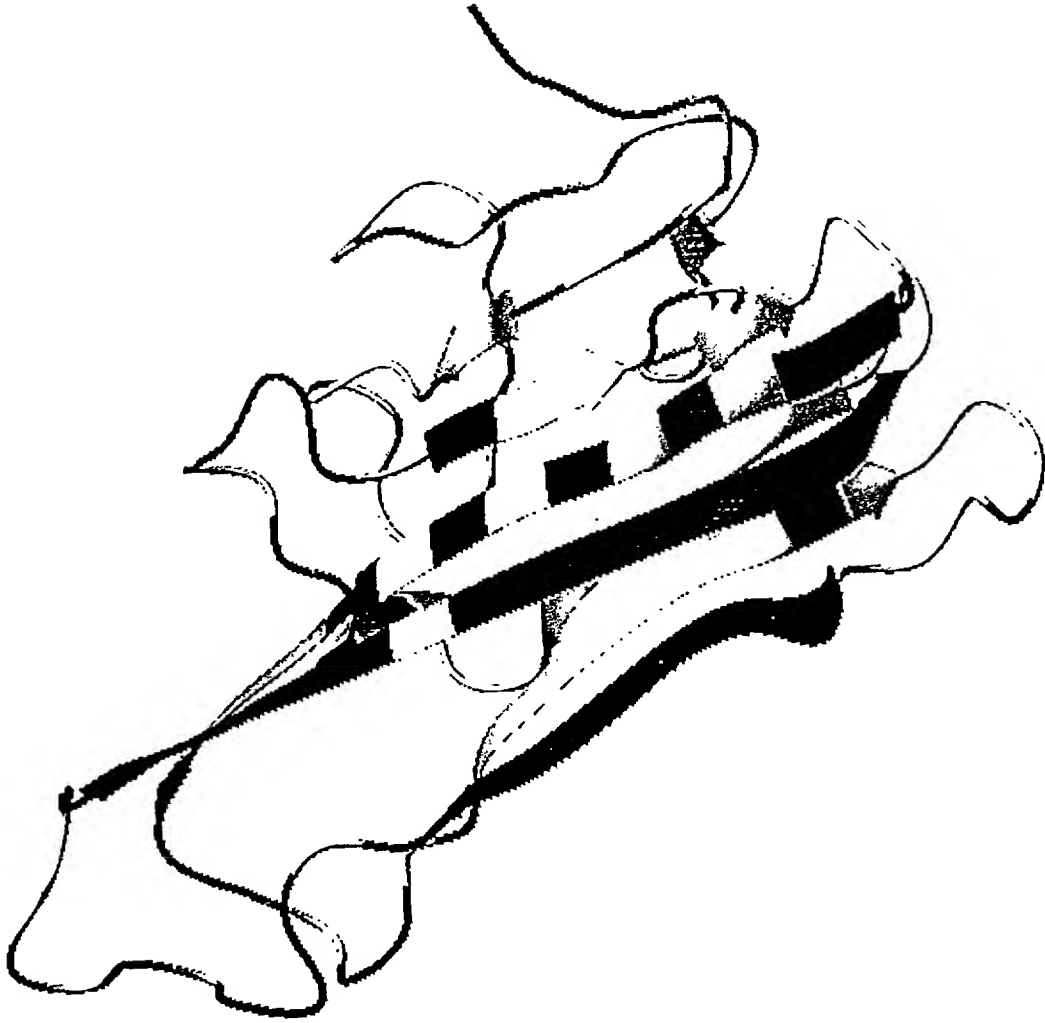


FIG._2

**FIG._3**

PDB ID: 1D51

TNF α BINDING SITES**FIG._4**

TNF α TRIMER INTERFACE**FIG._5**

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1   atgcaccacc accaccacca cgtacgctcc tcctcccgca ctccgctcga caaacccgta
61  gctcacgtag tagctaaccg gcaggctgaa ggtcagctgc agtggtgtaa ccgccgcgct
121 aacgctctgc tggctaaccg tgtagaactg cgcgacaacc agctggtagt accgtccgaa
181 ggtctgtacc tgatctactc ccaggtaactg ttcaaagggtc aggggtgtgccc gtccactcac
241 gtactgtgta ctacactatc ctcccgcatc gctgtatcct accagactaa agtaaaccgt
301 ctgtccgcta tcaaattcccc gtgtcagcgc gaaactccgg aagggtgctga agctaaaccg
361 tggtagaacg cgatctacct ggggtggtgta ttccagctgg aaaaagggtga ccgcctgtcc
421 gctgaaatca accgcccggg ctacctggac ttcgctgaat ccggtcaggt atacttcggt
481 atcatcgctc tgtga

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FIG._6A

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1   MHHHHHHVRS SSRTPSDKPV AHVVANPQAE GQLQWLNRRR NALLANGVEL RDNQLVVPSE
61  GLYLIYSQVL FKGQGCPSTH VLLTHTISRI 'AVSYQTKVNL LSAIKSPCQR ETPEGAEAKP
121 WYEPIYLGGV FQLEKGDRLS AEINRPDYLD FAESQVYFG IIAL

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FIG._6B

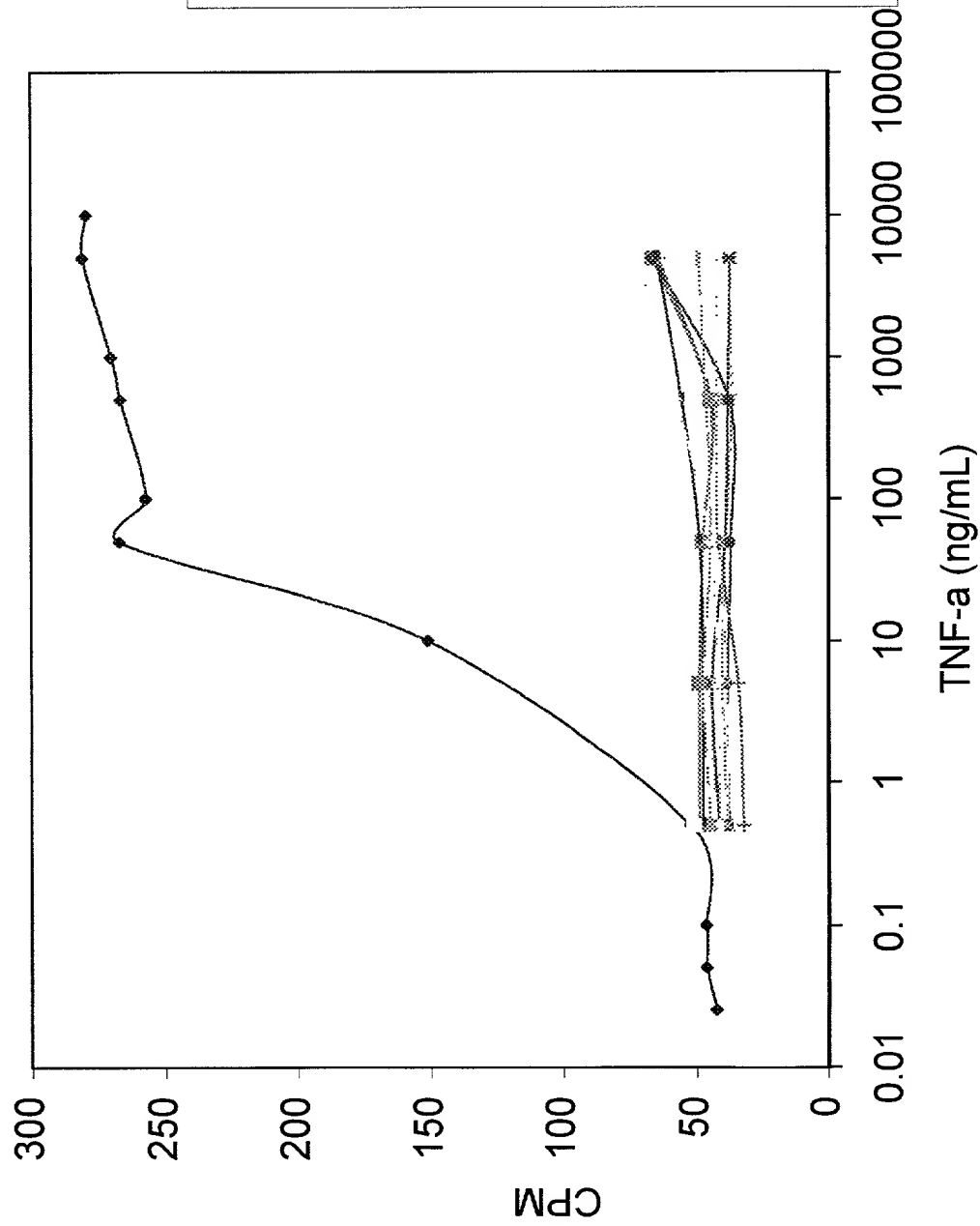
Wild-type TNF amino acid	Wild-type TNF amino acid number	Mutants created
Q	21	R
N	30	D
R	31	I, D, E
R	32	D, E, S
A	33	E
A	35	S
K	65	D, T, M, W, I, Q, S, N, V, E
G	66	Q, K
Q	67	D, W, Y, R, K, S
A	111	R, E
K	112	D, E
Y	115	Q, K, E, N, R, F, H, M, L, I, W, D, T, S
D	140	R, K
D	143	E, N, Q, S, R, K
F	144	N
A	145	R, D, K, N, H, T, Q, E, Y, M, S, F
E	146	N, K, R, S
S	147	R

ALSO MADE DOUBLE MUTANTS K65E/D143K, K65E/D143R, K65D/D143K AND K65D/D143R

FIG._7

T05T0T" 662T6669

Fig 8



—●— TNFα WT in House
 —◆— K112D
 —◇— Y115T
 —◇— D143K
 —*— D143R
 —●— Y115I
 —◆— D143E
 —●— A145R
 —◇— A145K
 —◇— A145E
 —◇— E146K
 —◇— E146R

Fig 9

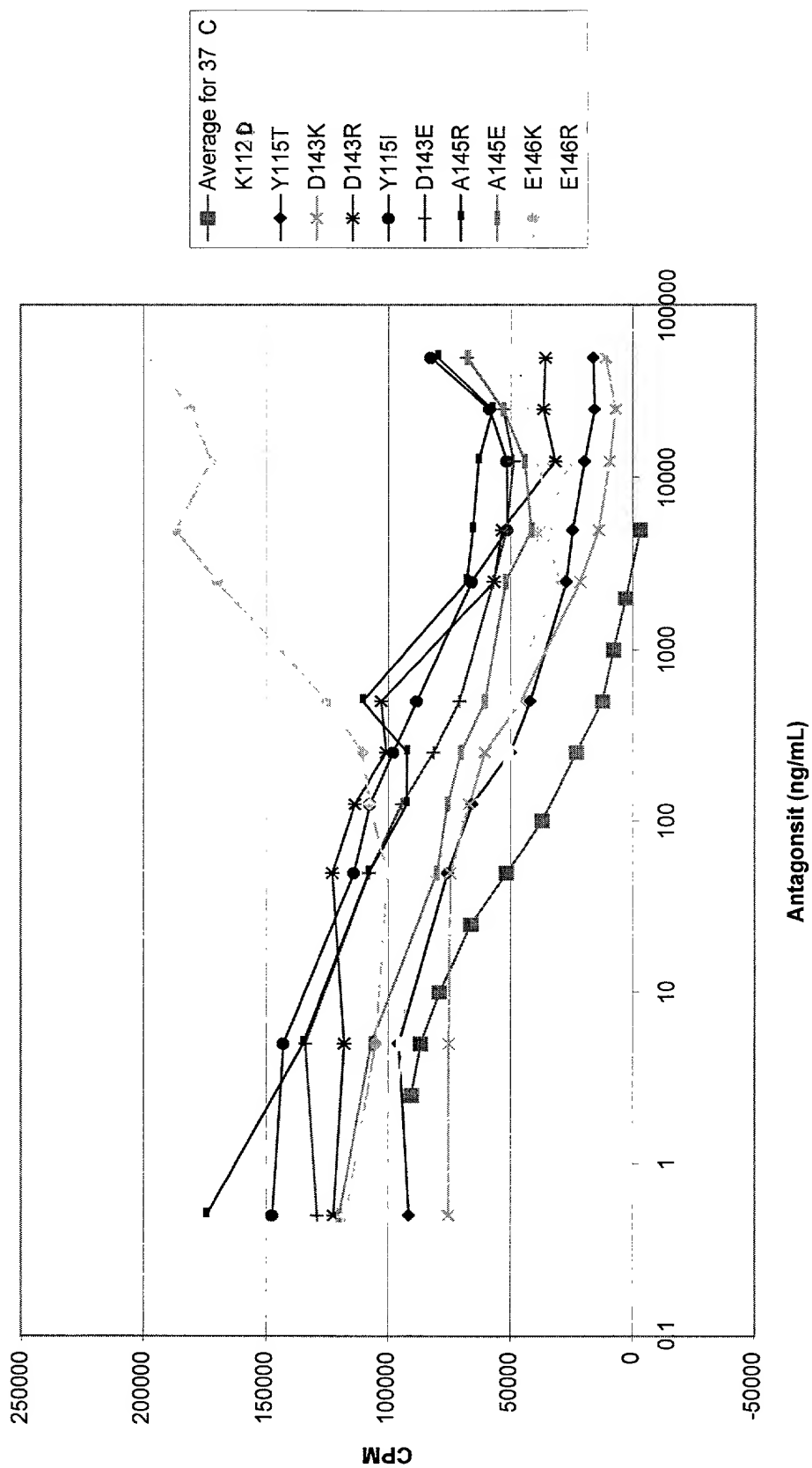


Fig 10a

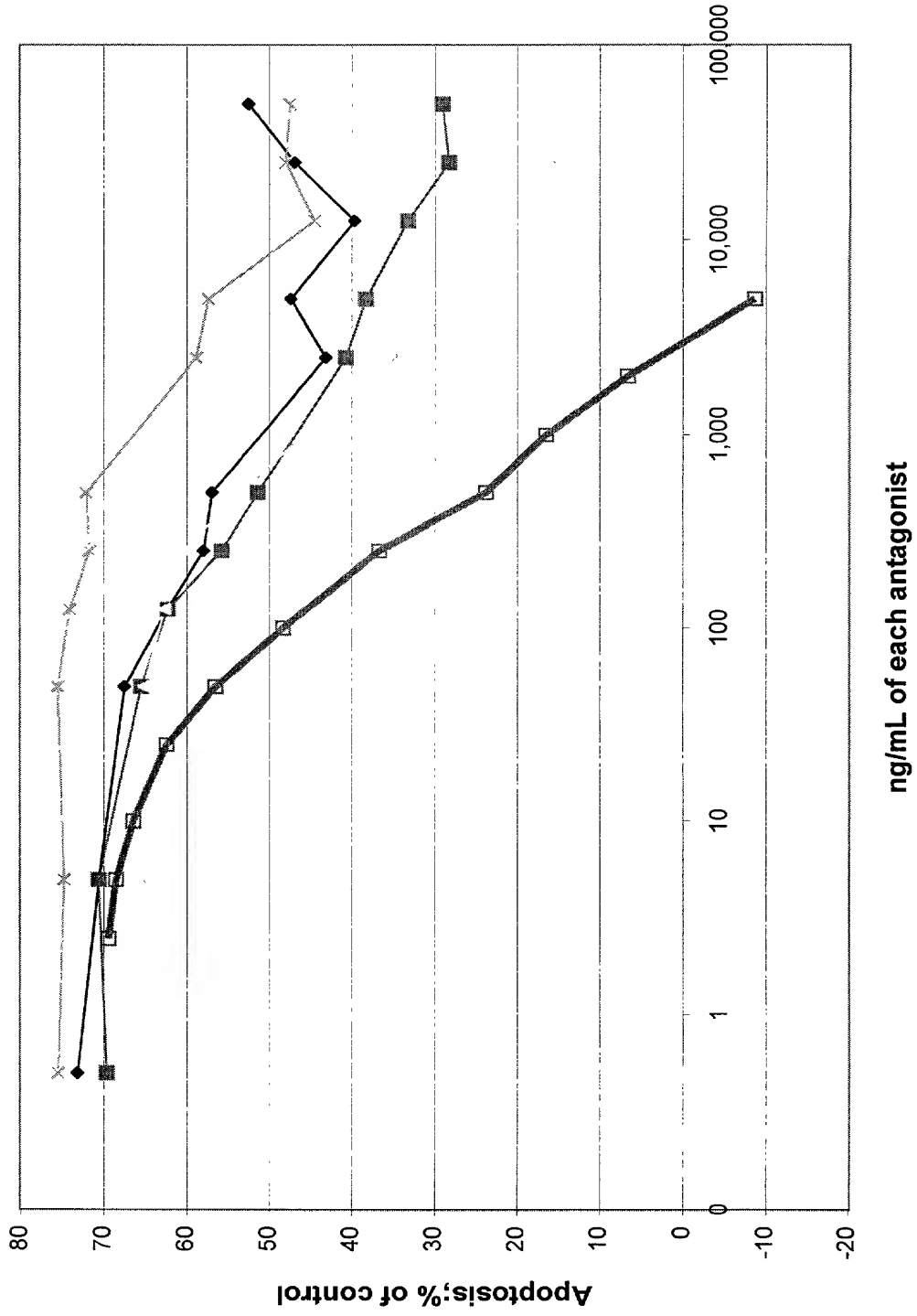
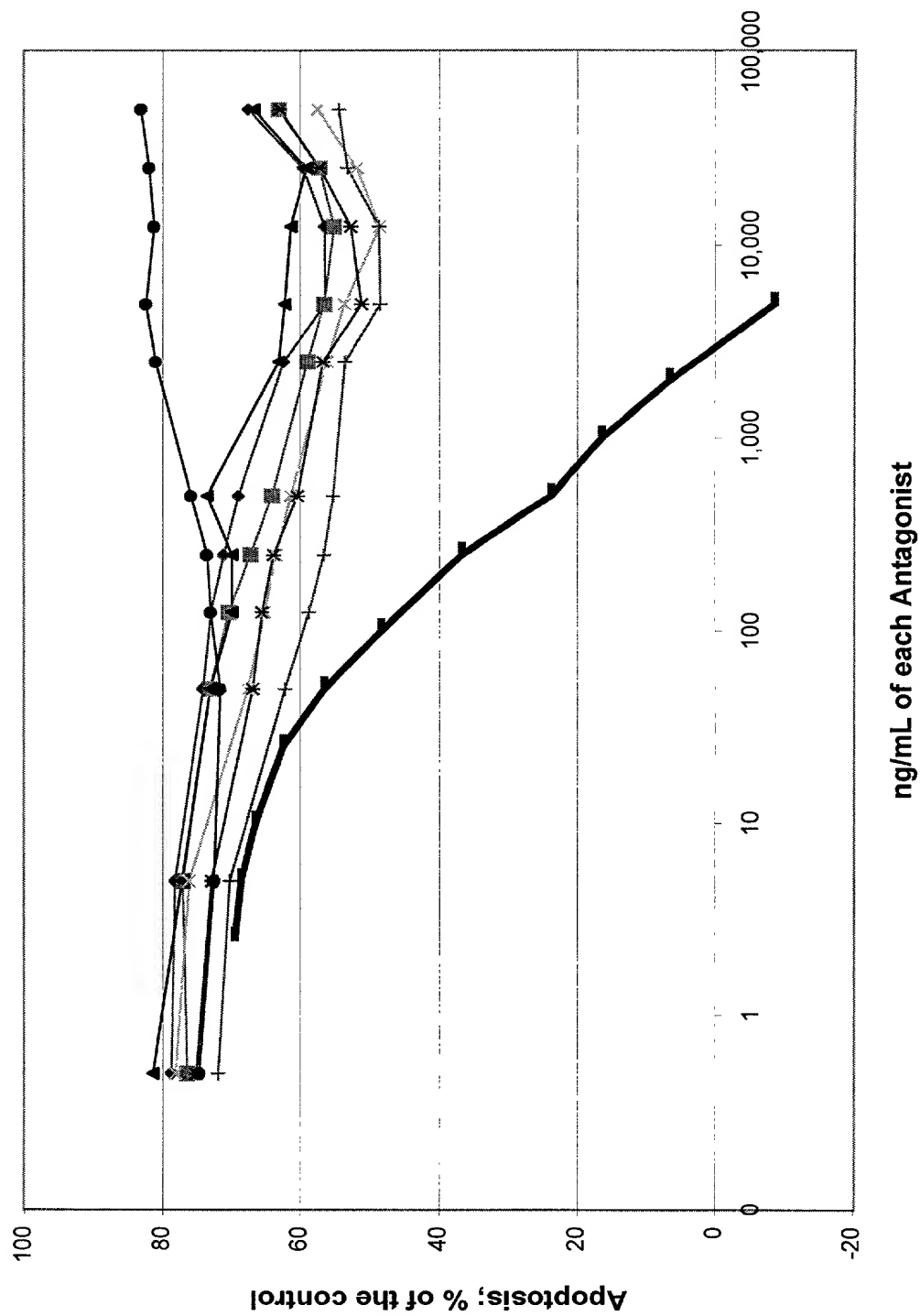


Fig-10b



FOSTOT 63213500

WT	PDA Relative Probability Distribution																		
Q21	R1000																		
N30	D1000																		
R31	I1000																		
R32	H1000																		
A33	E1000																		
A35	S1000																		
K65	R585	D146	K110	T42	H31	M27	W15	I15	Q10	S9	N9	V1							
G66	Q813	K187																	
Q67	D623	W209	Y83	R43	K41	S1													
A111	R959	E41																	
K112	K1000																		
Y115	Q230	K154	E116	N84	Y81	R72	F69	H43	M39	L36	I26	W25	D11	T8	S6				
D140	D1000																		
L143	D680	E130	N110	Q33	S29	R12	K6												
F144	F695	N305																	
A145	R456	D196	K124	N76	H67	T43	Q25	E9	Y1	M1	S1	F1							
E146	N489	K377	R111	D12	S10	E1													
S147	R1000																		

FIG._11

+

TRAF2(310-) DQDKIEALSSKVQQLERSIGLKDLAMADLEQKVLEMEA STYDG

FIG._12A

TRAF3(374-) VARNTGLLESQLSRHDQMLSVHDIRLADMDLRFQVLET ASYNG

FIG._12B

TRAF5(343-) NDQRLAVLEEETNKHDDTHINIHKAQLSKNEERFKLLEG TCYNG

FIG._12C

TRAF1(225-) DRERILSLEQRVVELQQTLAQKDQALGKLEQSLRLMEE ASFDG

FIG._12D

TRAF6(309-) QDHQIRELTAKMETQSMYVSELKRTIRTLEDKVAEIEA QQCNG

FIG._12E

TRAF4(201-) -----CALVSRQRQELQELRRELEELSV GS-DG

FIG._12F

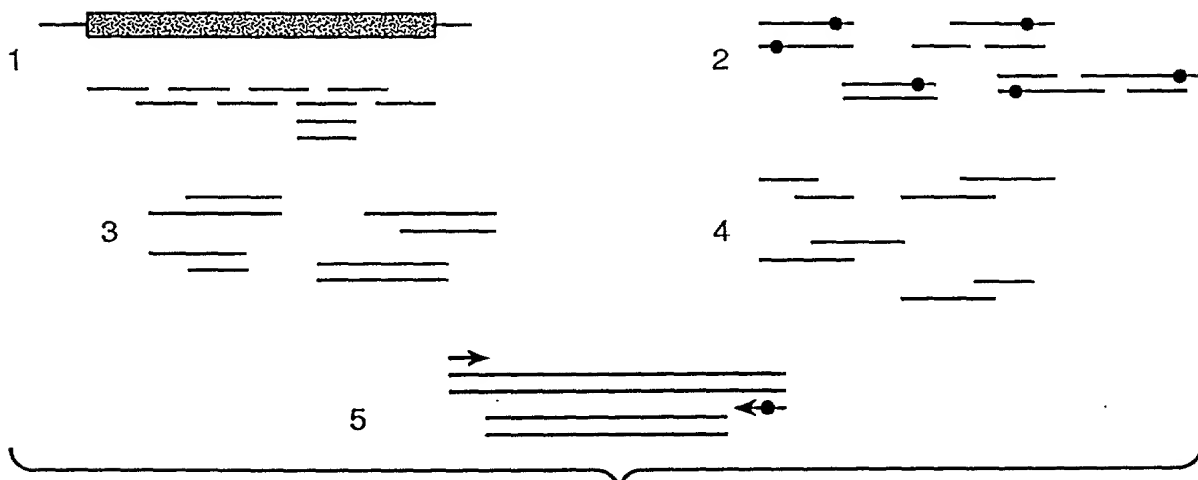


FIG._13

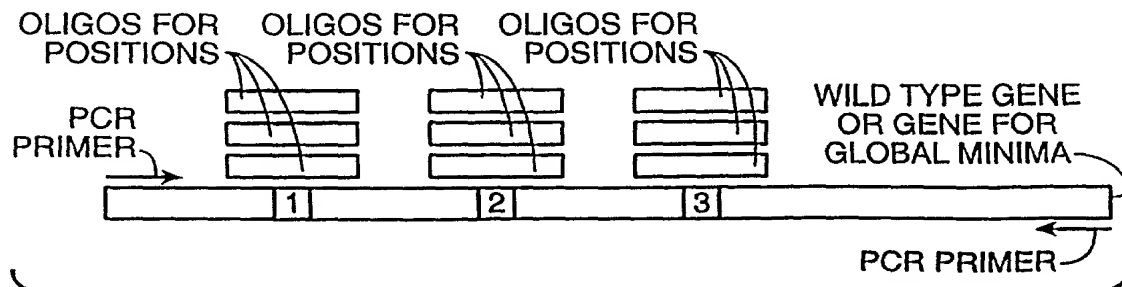
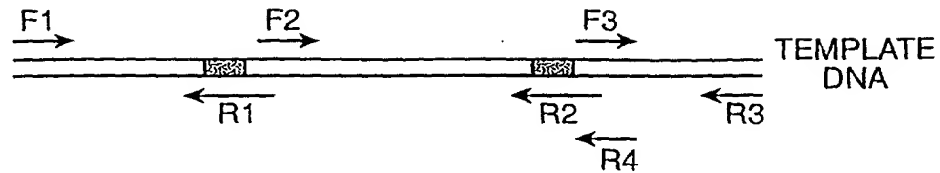


FIG._14

BLACK BOX =
REGION TO
BE MUTATED



STEP 1: SET UP 3 PCR REACTIONS:

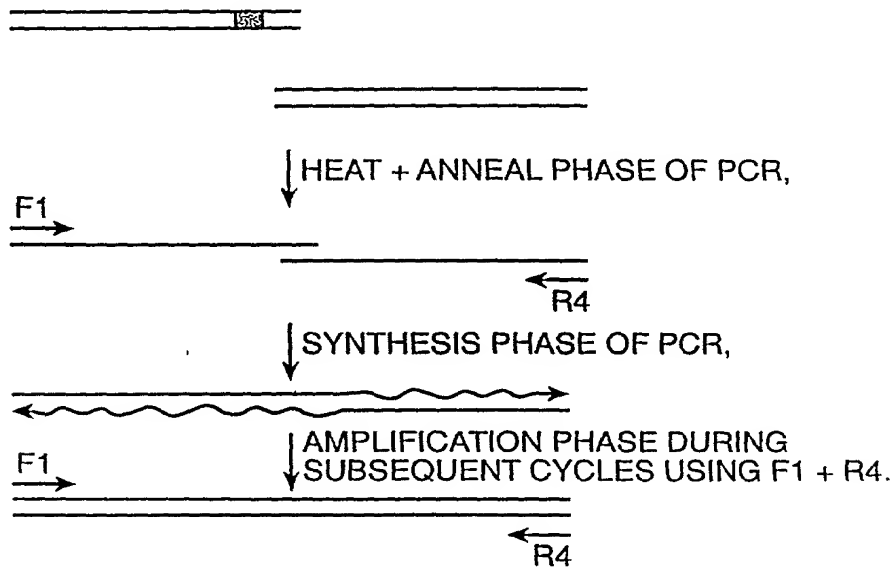
PRODUCTS:

TUBE 1:

TUBE 2:

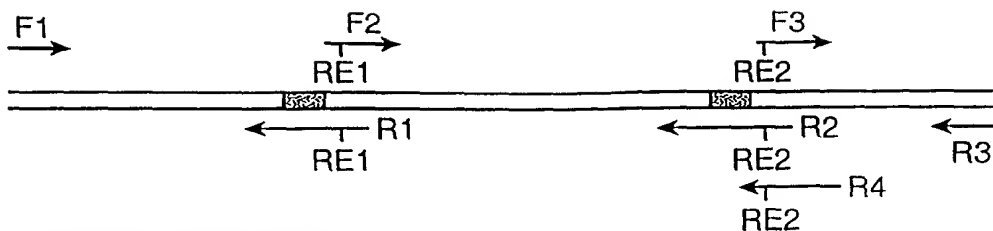
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 + PRODUCTS TUBE 2 + F1 + R4.



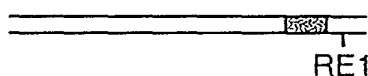
STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG. 15

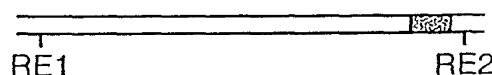


STEP 1: SET UP 3 PCR REACTIONS:

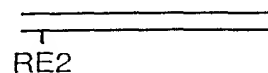
TUBE 1:



TUBE 2:



TUBE 3:



STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 1.



STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

FIG. 16

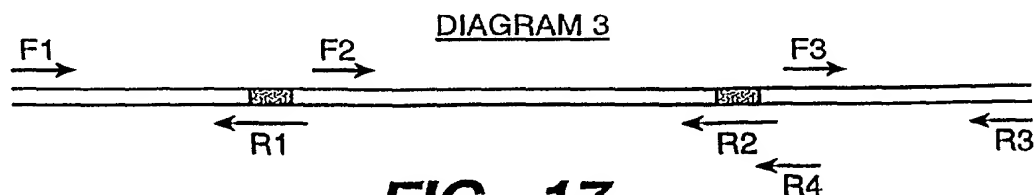


FIG. 17